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# Normal Human Cementum-Derived Cells: Isolation, Clonal Expansion, and In Vitro and In Vivo Characterization*

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## ABSTRACT

**Cultures of primary human cementum-derived cells (HCDCs) were established from healthy premolar teeth extracted for orthodontic reasons. Cementum was manually dissected, fragmented, and digested twice with collagenase. Following a thorough wash to remove liberated cells, the remaining cementum fragments were plated in Dulbecco's modified Eagle's medium/F12 medium containing 10% fetal bovine serum. Discrete colonies that contained cells exhibiting fibroblast-like morphology were visible after 14–21 days of culture. When the colonies became sufficiently large, cells from individual colonies were isolated and subcultured. Cementum-derived cells exhibited low levels or no alkaline phosphatase activity and mineralized in vitro to a lesser degree than human periodontal ligament (PDL) cells and human bone marrow stromal cell (BMSC) cultures. To study differentiation capacities of HCDCs, cells were attached to hydroxyapatite/tricalcium phosphate ceramic and transplanted subcutaneously into immunodeficient mice. The transplants were harvested 3, 6, and 8 weeks after transplantation and evaluated histologically. In human BMSC transplants, new bone tissue was formed with a prominent osteoblastic layer and osteocytes embedded in mineralized bone matrix. No osseous tissue was formed by PDL cells. Of six single colony-derived strains of HCDCs tested, three formed a bone-like tissue that featured osteocyte/cementocyte-like cells embedded within a mineralized matrix and which was lined with a layer of cells, although they were somewhat more elongated than osteoblasts. These results show that cells from normal human cementum can be isolated and expanded in vitro. Furthermore, these cells are capable of differentiating and forming mineralized tissue when transplanted into immunodeficient mice. (J Bone Miner Res 1998;13:1547–1554)**

## INTRODUCTION

CEMENTUM IS A MINERALIZED TISSUE that facilitates the attachment of periodontal ligament (PDL) to the tooth. Although the structure and the composition of cementum resembles bone, it is avascular and, under normal conditions, it does not undergo remodeling. The principal cells of cementum are cementoblasts, which line the surface of the tooth root, and cementocytes, which are embedded in the mineralized matrix in a manner similar to osteocytes.[1–3]

Due to the critical role of cementum in maintaining the structure of the periodontium and the high prevalence of periodontal disease, there is a great interest in the physiology of cementum. Establishing the in vitro model systems to study cell metabolism has greatly contributed to our understanding of the physiology of numerous tissues, including bone and cartilage. However, because of the size, topography, and small number of cells, there has been limited success in cementum cell biology. At present, the only in vitro models available employ cells either grown from benign tumors of cementum[4] or primary cultures of hetero-

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geneous cells derived from the PDL and cementum.[5–7] In the latter system, such mixed cellular populations were used as a source of immortalized cell lines. Recently, successful isolation and expansion of single cell–derived clones from these immortalized cell lines has been reported.[8] Cells obtained in this manner were positive for osteoblastic/cementoblastic markers, such as bone sialoprotein and osteopontin.[9–11] These two approaches, albeit successful in obtaining cellular growth, have some limitations, namely, utilization of either neoplastic or previously immortalized cells. Another strategy was based on isolation of cellular clones from nontransformed PDL-derived cell cultures.[6] Recently, Carnes et al.[12] have obtained cells from human cementum shavings. These cells showed alkaline phosphatase (ALP) activity and were positive for osteocalcin. However, in this system, similarly to the systems described above, it is unclear whether cells were derived from cementum and/or from the PDL.

To our knowledge, no studies have been performed to show conclusively the potential of any of these cells to form a mineralized matrix in an in vivo assay. Here we report the first successful isolation, in vitro expansion, and clonal selection of normal (i.e., nontransformed and nonimmortalized) primary human cementum-derived cells (HCDCs). Furthermore, to determine the nature of these cells, we have evaluated their mineralized matrix-forming capabilities employing an in vivo model of cell differentiation in immunodeficient mouse.[13–15]

## MATERIALS AND METHODS

### *Teeth*

Healthy human teeth (patient ages ranging from 12 to 14 years) extracted for orthodontic reasons ("human subjects" protocol approved by the Committee on Investigations Involving Human Subjects, School of Dentistry, University of North Carolina) were used. Teeth were either kept in serum-free Dulbecco's modified Eagle's medium (DMEM)/F12 medium (GIBCO BRL, Life Technologies, Grand Island, NY, U.S.A.) containing 100 U/ml of penicillin and 100 $\mu$g/ml of streptomycin (GIBCO BRL) overnight at 4°C or processed immediately after extraction, when possible.

### *Cell culture*

*PDL cell cultures:* PDL from the middle portion of the tooth root was scraped with a surgical scalpel, washed with DMEM/12 medium, and incubated in a medium containing collagenase P (Boehringher Mannheim, Mannheim, Germany), 100 mU/ml (10 ml of medium total/preparation) for 2 h at 37°C with rotation (25 rpm). The medium was collected, centrifuged at 500*g* for 10 minutes, and cells were washed three times with medium. After a final wash, cells were plated in DMEM/F12 containing antibiotics and 10% fetal bovine serum (GIBCO BRL). Cells were incubated in a humidified atmosphere, 95% air, 5% $CO_2$ at 37°C. Medium was changed every second day. Under the conditions employed, PDL cells grew without forming discrete colonies. When the cultures became semiconfluent (18–20 days in culture), cells were passaged with trypsin-EDTA (GIBCO BRL). PDL cells of second and fifth passages were used for experiments described below.

### *HCDC cultures*

The cultures of HCDCs were established from the same teeth as PDL cells using a modification of a method previously reported for the establishment of osteoblastic cell cultures.[16] After PDL was manually dissected from the tooth root with a surgical scalpel, each tooth was washed with DMEM/F12 medium and incubated in 10 ml of medium containing 100 mU/ml of collagenase P for 1 h at 37°C with rotation (25 rpm). The medium with released cells was discarded and the teeth were washed three times with fresh medium. Using a sterile surgical scalpel, cementum and a thin layer of underlying dentin were dissected and collected. We estimate (by histology and microscopic observations) that the average ratio of cementum to dentin was within the range of 1:3–1:5 and did not vary significantly between the different preparations. To minimize the variability in the relative cementum and dentin content within a group, fragments obtained from two to four teeth from the same patient were always pooled and processed together. Cementum/dentin fragments were thoroughly washed with medium (five times) and then minced with scissors until small fragments (<0.5 mm in diameter) were obtained. Fragments were washed with medium (five times) and then digested again with collagenase P (100 mU/ml; 4 ml total volume/0.1–0.2 ml of settled bed volume of fragments) for 1 h at 37°C (in some experiments, half of the sample was incubated for 1 h and the remaining half for 2 h). The medium with released material was discarded (in some experiments, the medium was collected, centrifuged at 500*g*, and the pellet was then resuspended and plated in Petri dishes to obtain cells from this fraction). The fragments were washed thoroughly (five times) with medium and then placed in 150 mm tissue culture plastic Petri dishes (Costar, Cambridge, MA, U.S.A.) containing 30 ml of growth medium (DMEM/F12 supplemented with penicillin/streptomycin and 10% fetal bovine serum). We have divided fragments obtained equally such that the number of cultures established from one patient was always identical to the number of teeth processed. On average, 0.05 ml of settled bed volume of fragments/plate was used. The cultures were incubated in a humidified atmosphere, 95% air, 5% $CO_2$ at 37°C for at least 5 weeks. Medium was changed every second day.

At the end of every step of this procedure, 5% of the fragments (settled bed volume) was fixed in Bouin solution and subjected to the routine histologic examination.

### *Isolation of clonal HCDC populations*

Since the cementum-derived cells formed colonies that were separated by large distances (see Fig. 1B), we isolated and expanded single-colony derived strains (SCDS) to obtain homogeneous populations of HCDC. When the cell number within a colony reached between 400 and 500, cells from individual colonies were scraped with a Pasteur pipette, transferred to the wells of a 12-well tissue culture plate

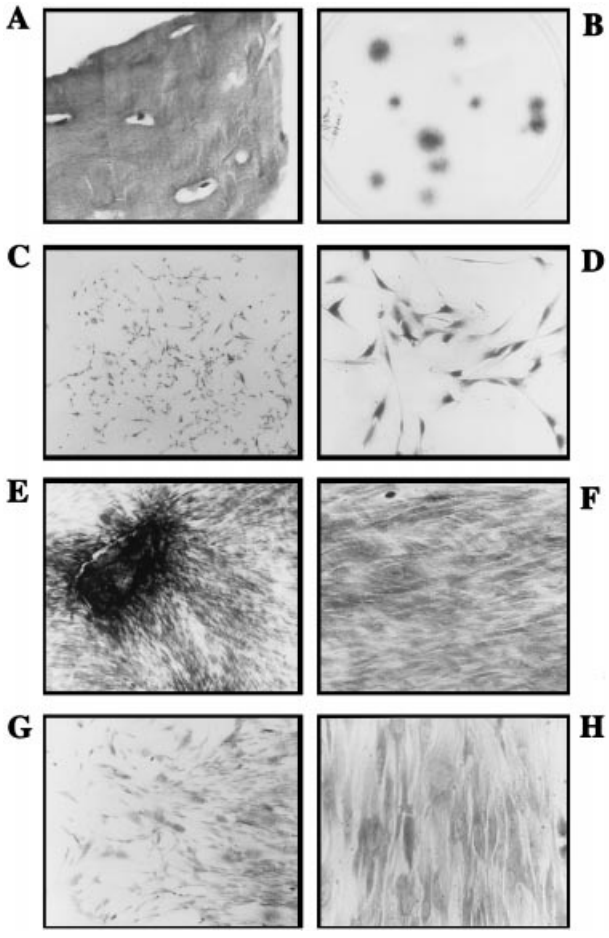


**FIG. 1.** (A) Microphotographic example of a cementum/dentin fragment used for establishing the cultures. Cementocytes are the only cells visible within the chip. Hemotoxylin and eosin staining, original magnification ×400. (B) Human cementum-derived cells in primary culture form distinct colonies. 6.5 weeks in culture. Crystal violet staining. (C, D) A typical colony of cementum-derived cells after 33 days in culture. Original magnification, (C) ×40 and (D) ×100. (E–G) A typical colony of cementum-derived cells after 46 days in culture. Cells in the middle of the colony became multilayered (E, F), particularly around remaining cementum/dentin fragments, seen here as a dark structure (E). Cells exhibit typical fibroblastic morphology with active cell migration and proliferation still observed at the edges of colonies (G, H). Original magnification, (E, G) ×100 and (F, H) ×400, crystal violet staining.

(Costar), and cultured in the growth medium. In this manner, about 10–15% of cells from an individual colony was transferred. Cells from the colonies that remained on the original plate were further incubated until semiconfluent and passaged, thus providing multicolony derived strains (MCDS).

### *Human bone marrow stromal cell cultures*

MCDS of human bone marrow stromal cells (BMSCs) were established from bone marrow content of normal spine bone fragments, as described.[14] The specimens used to initiate the cultures were obtained during the course of corrective surgery from a 15-year-old patient with scoliosis, under institutionally approved procedures for the use of human surgical waste.

### *In vitro characterization of cementum-derived cells*

Both MCDS and SCDS of HCDC were expanded in vitro until passage 2 or 3 and used for the in vitro and in vivo assays (see below). For the in vitro study, cells were plated into 48-well tissue culture plates (Costar) at the density of 30,000 cells/cm$^2$ and incubated in growth medium. Human PDL cells, human BMSCs, and normal skin fibroblasts (line CRL 1906, passage 11; ATCC, Rockville, MD, U.S.A.) were used for comparison. Upon reaching confluence, cells were incubated in the differentiation medium (DMEM/F12 medium containing 50 $\mu$g/ml ascorbic acid (Sigma, St. Louis, MO, U.S.A.), 2.5 $\mu$g/ml of insulin-transferrin-selenium (ITS; Boehringer Mannheim), 10 mM beta-glycerophosphate (Sigma), and $10^{-8}$ M dexamethasone (Sigma). Following 14 days of incubation, cells were evaluated for ALP activity (kit 86-R; Sigma) according to the manufacturer's protocol. For mineral deposition in vitro, the von Kossa staining method was employed.[17] Each assay was performed in triplicate.

### *In vivo differentiation assay*

Cementum-derived cells (both SCDS and MCDS) were assessed for their potential to form mineralized matrix upon transplantation into immunodeficient mice. This model is well established and it has been demonstrated in this system that the tissue formed by transplanted cells is of donor origin and that the hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic used as a carrier is permissive for several phenotypes (bone, fibrous tissue, marrow stroma, adipose tissue).[13–15] Thus, this in vivo assay offers an excellent screening tool for testing the commitment of transplanted cells toward a particular phenotype. Human PDL cells as well as human BMSCs were used for comparison. Cells from the second, third, and fifth passage were used. For a single transplant, 40 mg of HA/TCP ceramic powder (Zimmer, Warsaw, IN, U.S.A.) was mixed with $1.5 \times 10^6$ cells. Trypsin-released cells were centrifuged in 1.7 ml microtubes at 135*g* for 10 minutes, resuspended in 1 ml of growth medium, and mixed with the ceramic powder. Cells were next incubated at 37°C for 90 minutes with slow rotation (25 rpm), centrifuged briefly, and the pelleted HA/TCP powder with adherent cells was transplanted subcutaneously into 8- to 12-week-old female beige mice (NIH-bg-nu-xidBR; Harlan Sprague Dawley, Indianapolis, IN, U.S.A.) as described.[13–15] Briefly, mice were anesthetized by intraperitoneal injection of 2.5% tribromoethanol (Sigma) at 0.018 ml/g of body weight. Single 1-cm-long skin incision was made on the dorsal surface of each mouse, and four subcutaneous pockets per mouse were created by blunt dissection. A single transplant was placed into each pocket, and incisions were closed with surgical staples. All procedures were performed under institutionally approved guidelines for the use of animals in research.

The transplants were harvested 3, 6, and 8 weeks after transplantation. Transplants were cut in half, fixed, and partially decalcified for 2 days in Bouin's solution (Sigma) and then processed for routine histologic examination (hematoxylin-eosin staining). On average, two to four transplants containing identical cells were evaluated, yielding consistent results.

## RESULTS

### *Cementum-derived cell cultures*

Of the 15 attempts to obtain cementum cell cultures, 6 were successful, i.e., cellular outgrowth was obtained after 3 weeks of incubation. Three of the cultures were terminated within a few days due to yeast contamination, and six cases demonstrated no cell outgrowth after 5 weeks. Teeth that were processed immediately after the extraction yielded cellular outgrowth more frequently (four out of six) than teeth that were kept overnight prior to the procedure (two out of six attempts). We believe that the failure to obtain cellular growth from some of the preparations was likely due to the fact that the cellular cementum was less abundant in some of the teeth used and/or all cells with proliferative potential were lost during storage, or during the procedure.

Histologic examination of the cementum/dentin fragments after two collagenase P treatments demonstrated that very few cells were present in the material used for establishing the cultures, and the only cells remaining were within the fragment, indicating that they were indeed cementocytes (Fig. 1A). No cells were visualized at the surface of the chips (where remnants of PDL might have been present), and no cells were detected within the dentin underlying the cementum. Thus, we tentatively have concluded that cementocytes that had migrated from the cementum fragments and proliferated were most likely the source of the cells in our culture system.

Incipient colony formation was observed 14–20 days after plating. Each colony was labeled on the outside of the dish and the development of each colony was monitored daily using an inverted microscope. On average, 10–12 colonies per tooth exhibiting sustained growth were obtained (Fig. 1B) when teeth had been kept overnight prior to the procedure and 18–21 colonies per tooth were obtained when teeth had been processed immediately after extraction. In the latter case, colony formation occurred earlier, usually around day 14, while it took 17–21 days for the former. A few days prior to definitive colony formation, individual fibroblast-like cells were observed, indicating that each colony was formed by proliferation of a single cell that had migrated from the fragment. No new colony formation was detected after day 25 until day 32–35 of culture. This late colony formation we attribute to the "carry-over" of cells from an established colony that had become attached to a cementum/dentin fragment that moved to a new location.

Interestingly, fragments obtained from freshly extracted teeth incubated in collagenase for 2 h yielded more extensive colony formation (30 colonies/tooth) than those incubated for 1 h only (18 colonies/tooth). We speculate that the prolonged collagenase treatment may facilitate cellular migration out from the chip, because the unmineralized tissue surrounding the cells is removed more efficiently and more space around the cell is created.

When the medium containing cells released by the second collagenase treatment was plated, only once have we detected a single colony of fibroblastic cells (out of four attempts from the same preparations that yielded growth of cementum-derived cells from the chips). If no cellular outgrowth was detected in the dishes containing the cementum/dentin fragments, no cells grew from the material released by the second collagenase digestion.

The cells within colonies showed typical fibroblastic morphology (Figs. 1C–1H) and were similar to both human BMSCs and PDL cells in comparable cultures (compare cells in Figs. 2 and 3A). In 5-week-old cultures, the colonies consisted of several hundred to one thousand cells (Figs. 1C and 1D). Based on the microscopic observations, we estimated that the average population doubling time was between 27 and 30 h. Later (7–8 weeks in culture), colonies became multilayered, and some exhibited nodule-like structures, particularly around the fragments of cementum/dentin (Figs. 1E–1F). At this stage, active cell proliferation and migration were observed mostly on the edge of the colonies (Figs. 1G and 1H).

### *Isolation and partial characterization of clonal cell populations*

When colonies of cementum-derived cells reached the size of 400–500 cells/colony (around 35 days in culture for cultures established from teeth kept overnight), we isolated cells from individual colonies and further expanded them in vitro. In 90% of the cases, cellular growth of transferred cells was maintained for two or more passages.

SCDS of HCDCs were further expanded in vitro. Each strain was divided in half. One half was subjected to in vivo transplantation and the other half was characterized in vitro for ALP activity and mineral deposition in culture (Fig. 2 and Table 1). Both early (2nd and 3rd) and late (5th and 6th) passages of HCDCs and control cells (PDL cells and BMSCs) were tested. All of these cell populations markedly resembled each other during routine culturing, and extended passaging did not significantly alter their morphology. When maintained in regular growth medium (without ascorbate, dexamethasone, and $\beta$-glycerophosphate), only the BMSC cultures showed detectable ALP activity (Table 1). Following a 2-week incubation in differentiation medium (supplemented with ascorbate, dexamethasone, ITS, and $\beta$-glycerophosphate), only SCDS clone #2 and MCDS of HCDCs showed weak but detectable and reproducible ALP activity. In contrast, PDL cells and BMSCs exhibited high numbers of cells positive for ALP (Table 1 and Fig. 2). Detectable levels of ALP activity were also present in skin fibroblast cultures when cells were maintained in differentiation medium (Table 1). Most of the cultures, including skin fibroblasts and PDL cells, showed mineral deposition via von Kossa staining following a

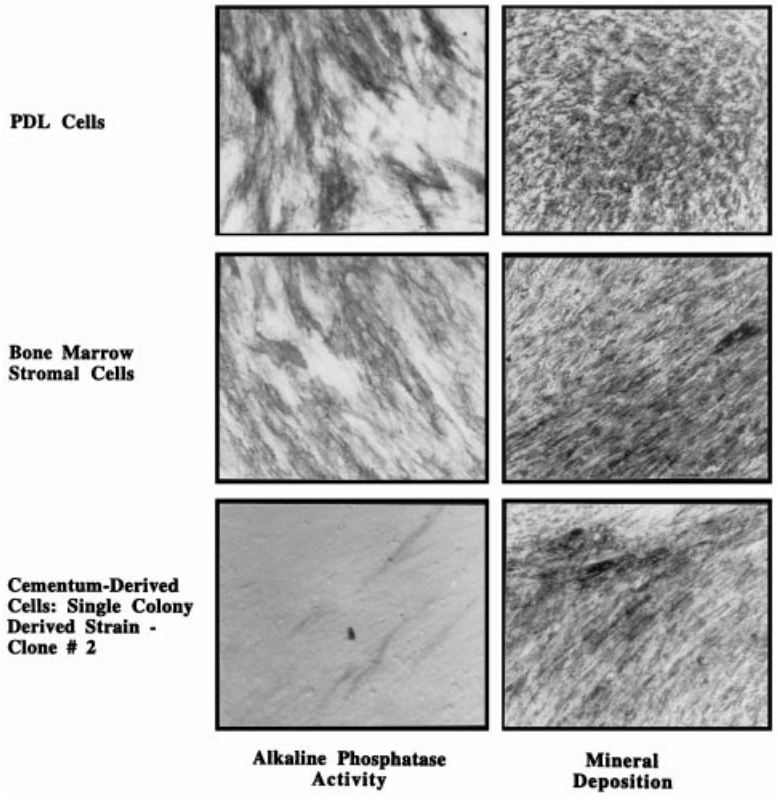


**FIG. 2.** ALP activity and mineralization in vitro of PDL cells, BMSCs and SCDS of human cementum-derived cells. Second passage of cells, original magnification, ×100.

2-week incubation in differentiation medium (Table 1 and Fig. 2).

### *In vivo differentiation assay*

Six to eight weeks post-transplantation, mineralized tissue was formed in transplants of three out of six SCDS, as well as in transplants of MCDS of HCDCs (Table 1 and Fig. 3). At earlier time points (3 weeks after transplantation), only one SCDS out of six showed detectable mineralized matrix formation. These results were obtained for cells at passage 2 or 3. We have further tested cells from the fifth passage of the two SCDS of HCDCs that had been cemento/osteogenic at passage 3. One of these strains had lost its capability, while the other maintained its ability to form mineralized matrix. BMSCs formed bone, as expected, while PDL cells did not form any detectable mineralized matrix within transplants at any time points of observation (Table 1 and Fig. 3) regardless of passage number (from 2 to 5). Since no cementum-specific markers are known at present, we cannot provide definitive proof that the tissue formed by HCDCs was indeed cementum. Thus, we will further refer to this tissue as mineralized matrix.

Histologically, discrete differences were observed between tissues formed by cementum-derived cells and BMSCs. First, cementum cell-derived tissue consistently appeared to be less cellular than that formed by BMSCs (Fig. 3C). Second, in most transplants of HCDCs, the cells lining the newly deposited mineralized matrix were consistently larger and more elongated than corresponding cells in transplants formed by BMSCs (Figs. 3B and 3C). In some sections, fibrous tissue reminiscent of PDL was present, suggesting that HCDCs can differentiate into PDL, and/or the deposited mineralized matrix may induce the formation of a PDL-like structure (Fig. 3).

## DISCUSSION

We report here the successful isolation, in vitro expansion, and establishment of SCDS of normal human cementum-derived cells. Furthermore, we have conclusively shown that both SCDS and MCDS maintain their potential to form mineralized matrix after ex vivo expansion, as they form cementum/bone-like tissue upon transplantation in vivo.

The method for obtaining cementum cells described in this study was designed to ensure that only cells that are protected by mineralized extracellular matrix were present in the starting material at the time that the cultures were initiated. Previously, a similar strategy had been successfully employed for obtaining human bone cells.[16] We were unable to detect any cells other than cementocytes in the fragments that were used for establishing the cultures. No cells or cell remnants were detected within the dentin underlying cementum, as expected. Because prolonged culture incubation (up to 3 weeks) was required before any cells became visible, cementocytes were most likely the sole source of cells in our system. In fact, PDL cells in parallel cultures were readily visible after only several days of incubation. Although we cannot exclude the possibility that other cells, including cementoblasts and/or PDL cells

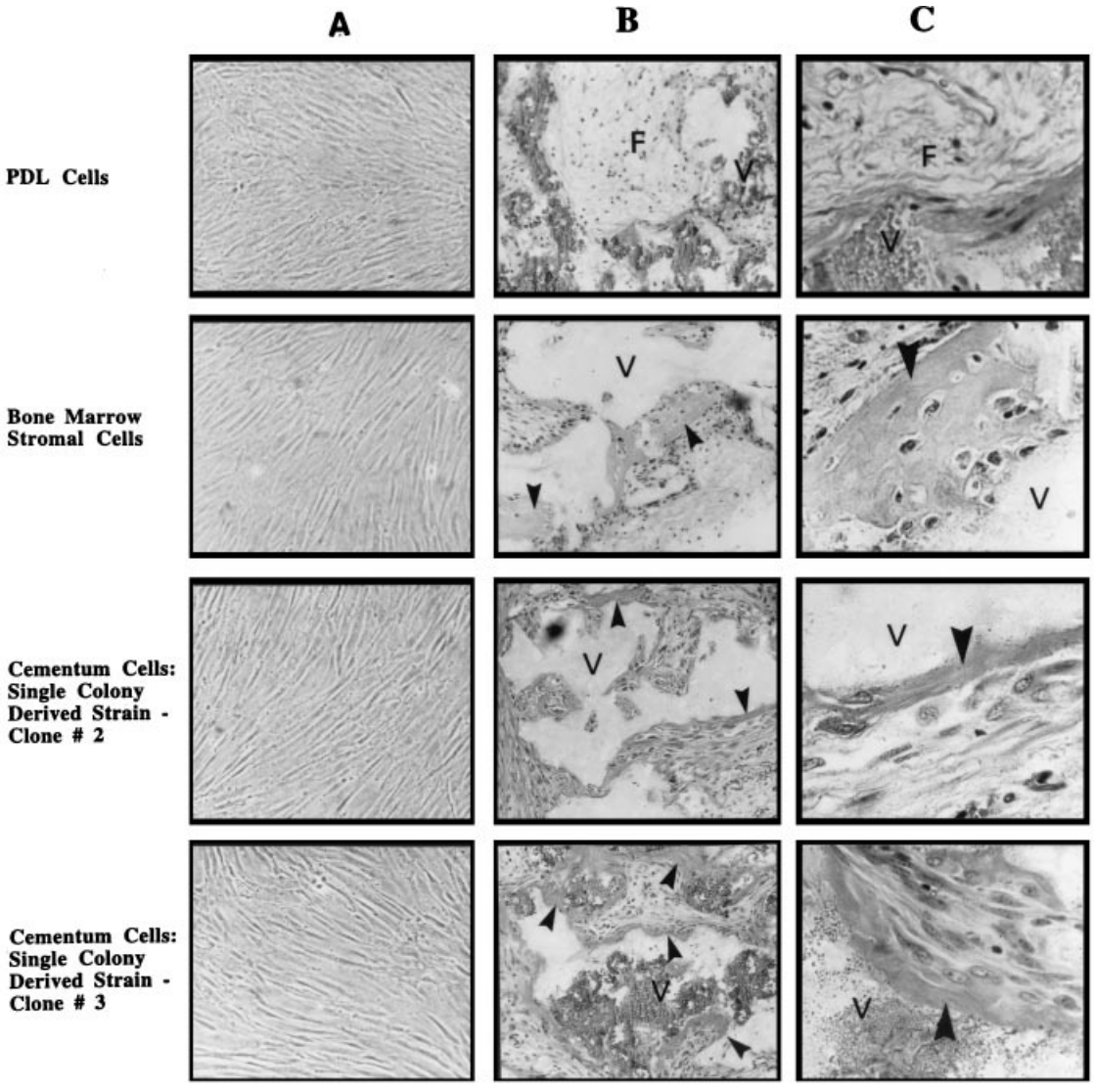


**FIG. 3.** (A) In vitro morphology of PDL cells (passage 5), BMSCs (passage 2), and two representative SCDS (both passage 3) of human cementum-derived cells prior to transplantation. Original magnification, ×100. (B, C) Morphology of the representative transplants recovered from recipient animals. (B) Original magnification, ×100; (C) original magnification, ×400. For PDL and clone #3 of SCDS of cementum-derived cells, the histology of transplants harvested at 6 weeks after transplantation is shown; for BMSC and clone #2 of SCDS of HCDCs, grafts were harvested 3 weeks after transplantation. v = vehicle (hydroxyapatite/tricalcium phosphate); *F* = fibrous tissue; arrowhead = bone/cementum-like tissue.

closely associated with cementum might have escaped detection, we believe it highly unlikely, considering the stringency of collagenase treatments. Furthermore, more colonies were obtained following prolonged incubation in collagenase, and only once was a single colony obtained (out of four attempts) from the medium containing material released by the second collagenase digestion. These observations provide further support for the notion that all of the cells residing within unmineralized PDL matrix (and not protected from collagenase digestion) adjacent to the cementum were removed by manual cleaning and by collagenase treatments and indirectly show that cells from PDL did not contribute to the cultures of cementum-derived cells in any significant manner. The fact that MCDS of PDL cells did not form mineralized matrix in an in vivo assay (in contrast to MCDS of HCDC) also argues against PDL cells being present in the HCDC cultures.

If some populations of cementocytes indeed retain the capability to migrate and proliferate as our results suggest, then cementocytes may not necessarily be terminally differentiated cells, as has been generally assumed.[2,3] Furthermore, if cementocytes can be stimulated to proliferate and migrate in vivo, then they may be possible target cells for therapies aimed at cementum regeneration.

Cementum cells are thought to be closely related to osteoblasts and to some extent our data support this view.

Table 1. The Results of In Vitro and In Vivo Assays

| Cells | *Alkaline phosphatase activity* | | *Mineralization in vitro* | *Cementum/bone-like tissue formed in vivo* |
|---|---|---|---|---|
| | *UND* | *DIFF* | | |
| HCDC SCDS clone #1 | − | ± | ++ | ± |
| HCDC SCDS clone #2 | − | + | ++ | F |
| HCDC SCDS clone #3 | − | − | + | F |
| HCDC SCDS clone #4 | − | − | ± | NF |
| HCDC SCDS clone #7 | − | ± | ++ | F |
| HCDC SCDS clone #8 | ND | ND | ND | NF |
| HCDC SCDS clone #9 | − | − | ++ | ND |
| HCDC SCDS clone #10 | − | − | + | ND |
| HCDC SCDS clone #11 | − | − | + | NF |
| HCDC MCDS | − | + | + | F |
| PDL cells | − | ++++ | +++ | NF |
| Skin fibroblasts | − | + | + | ND |
| BMSC | ++ | ++++ | +++ | F |

HCDC, cementum-derived cells; SCDS, single colony derived strain; MCDS, multicolony derived strain; BMSC, bone marrow stromal cells; PDL, periodontal ligament.

UND, cells maintained in growth medium; DIFF, cells maintained in differentiation medium for 2 weeks.

++++, strongly positive; +++, positive; ++, moderately positive; +, weakly positive; ±, uncertain; −, negative; ND, not determined.

F, mineralized matrix formed; NF, mineralized matrix not formed, ±, uncertain.

These cells exhibit fibroblastic morphology in culture and form bone-like tissue after transplantation, similar to BMSC. However, cementum-derived cells showed significantly different phenotypic features compared with BMSCs in culture, particularly in the in vitro differentiation assay. While both BMSCs and PDL cells showed high ALP activity, most of the HCDCs did not. There are suggestions that lower ALP activity is indeed indicative of cementoblastic phenotype in vivo.[6,18] It is also possible that longer periods of incubation may be required for HCDCs to become fully differentiated in vitro, and/or conditions for such transition are different from those required by PDL cells and BMSCs. While it is still premature to draw any definite conclusions about the relationship between cementum-derived cells and osteoblastic cells, our in vitro studies suggest that likely they are not identical.

Of the several SCDS of HCDCs tested in vivo, half formed cementum/bone-like tissue. Although this represents a limited number of cultures, it is intriguing that approximately the same percentage of SCDS of human BMSCs form bone after transplantation in vivo.[14] Our results also show that not all cell strains that can be expanded from cementum exhibit the committed cementum/osteogenic phenotype, and, furthermore, extended passaging may result in the loss of such commitment. It also appears that some of the SCDS of cementum-derived cells form mineralized matrix at a slower rate than BMSCs. In several cases, no mineralized matrix was present when transplants were harvested 3 weeks after transplantation, but at the later time points (6 and 8 weeks after transplantation) the same strains tested positive in this assay. This appears to be consistent with the differences in the general dynamics of bone and cementum formation in situ.

As mentioned above, at present there are no molecular markers specific for cementum cells. Based on the in vitro and in vivo results obtained in this study, we propose that only the cellular strains of HCDCs that are capable of forming mineralized tissue after transplantation can be used as a model system to study cementum cells in vitro. Such a system may offer a tool for searching for cementum-specific biochemical markers, if any, that would allow to distinguish cells committed to the cementoblastic/cementocytic phenotype from cells committed to the osteoblastic/osteocytic phenotype.

Interestingly, no mineralized tissue was formed in transplants carrying cells derived from PDL. This was a somewhat unexpected finding, as it has been generally accepted that osteoblastic/cementoblastic precursors are present in the PDL.[6,19–25] However, the evidence supporting this notion came exclusively from in vitro studies, showing that some of the PDL-derived cells can express osteoblastic markers (ALP, bone sialoprotein, and osteopontin expression, mineralization in vitro). Judged by these parameters, PDL-derived cells employed in this study fulfillled the criteria for a cemento/osteoblastic phenotype and yet they did not form any mineralized tissue after transplantation. If our observation proves to be valid following more extended studies, then apart from the obvious conclusion that there are no committed osteogenic/cementogenic precursors present in normal conditions in the PDL, several alternative explanations are possible. First, committed osteogenic precursors could be simply lost early in the culture and the remaining cells (regardless of the apparent in vitro phenotype) are not capable of following this differentiation pathway in vivo. Second, osteogenic cells in the PDL may be under negative regulation by other fibroblastic populations (we used PDL cultures containing heterogeneous fibroblas-

tic populations) that exert a suppressive influence in vivo.[24,25] Thus, some of the SCDS from PDL may be cemento/osteogenic, while the mixed population is not. Third, the osteogenic precursors in PDL may not be committed, but may require an inducing stimulus (e.g., BMPs) and will form mineralized tissue only when this stimulus is applied. Clearly these fundamental questions need to be addressed, and the combined in vitro/in vivo approach would provide invaluable means for that purpose. Results obtained so far, however, suggest that PDL may not be a good source of cells to study osteo/cementoblastic differentiation, even though these cells in vitro are capable of expressing some markers associated with the osteoblastic phenotype.

In conclusion, we have established a method for culturing nontransformed, nonimmortalized human cementum-derived cells in vitro. We have also isolated and expanded in vitro SCDS of such cells and partially characterized them in vitro. We have shown that some of these strains represent a pool of cells committed to the formation of mineralized matrix in an in vivo transplantation assay. We propose that until defined, cementum cell-specific markers are developed, cellular strains of HCDCs need to be tested for the potential to form mineralized matrix following transplantation and only the strains that maintain such potential can be considered "cementum-derived cells" in vitro. The system described here may provide an invaluable tool to study cellular populations and molecular mechanisms of cementogenesis. In addition, our results underscore the value of combined in vitro/in vivo approach when developing new cell culture systems and interpreting data derived from them.

## ACKNOWLEDGMENTS

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